Behavioral Ecology

The official journal of the ISBE International Society for Behavioral Ecology

Behavioral Ecology (2014), 25(3), 668–674. doi:10.1093/beheco/aru043

Original Article

Cheating workers with large activated ovaries avoid risky foraging

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Received 4 October 2013; revised 12 February 2014; accepted 17 February 2014; Advance Access publication 22 March 2014.

Eusocial insects undoubtedly evolved from solitary ancestors, but how this occurred is not well established. The Ground Plan hypothesis suggests that gene networks that once regulated the oviposition and foraging phases of an ancestral solitary insect's life cycle have been co-opted to establish the queen–worker dimorphism in extant eusocial insects; queens permanently express genes that were once expressed during the oviposition phase, whereas workers express genes that were once associated with foraging. An extension of the Ground Plan hypothesis, the Reproductive Ground Plan-forager hypothesis, proposes that foraging specialization by worker honey bees for either pollen or nectar is controlled by the same reproductive gene networks. According to the Reproductive Ground Plan-forager hypothesis, workers with more ovarioles forage early in life and specialize in pollen collection. Here we find that among workers of a highly reproductive honey bee subspecies, *Apis mellifera capensis*, there is a positive correlation between ovariole number and age at onset of foraging, and no association between ovariole number and foraging preference, thus contradicting key aspects of the Reproductive Ground Plan-forager hypothesis. We also find a negative association between ovariole number and ovary activation, suggesting that high ovariole number is not directly related to reproductive potential as previously assumed.

Key words: ***Apis mellifera capensis*, eusociality, foraging specialization, Reproductive Ground Plan hypothesis.**

INTRODUCTION

Eusocial insects are those species in which there is reproductive division of labor between a queen and worker caste (Wilson and Hölldobler 2005; Beekman et al. 2006). The "ovarian" (West-Eberhard 1996) or "bivoltine" (Hunt and Amdam 2005) Ground Plan hypothesis provides a plausible explanation of the evolutionary transition from a solitary life history to one of eusociality. In some solitary wasp species, the life history of females involves cycling between reproductive and foraging phases. During the foraging phase, solitary females build a nest and forage for proteinacious food with which to provision brood cells. During this phase, the females have inactive ovaries. After the brood cells are constructed, the female ceases foraging, activates her ovaries, and lays eggs. The female may then seal the brood cells and reenter a cycle of foraging and nest construction (West-Eberhard 1989, 1996; Hunt 2007). A transition to sociality could therefore arise if the cycling of behaviors that typify some solitary insects became decoupled so that some individuals are locked in the foraging phase and became workers while others were locked in the reproductive phase and became queens (West-Eberhard 1989, 1996; Gadagkar 1996; Hunt 2007). This hypothesis has been termed the "Reproductive Ground Plan hypothesis" (Amdam et al. 2004).

In recent years, the Reproductive Ground Plan hypothesis (Amdam et al. 2004) has been extended to explain foraging specialization of workers in the honey bee *Apis mellifera*. Honey bee workers tend to specialize in gathering nectar, pollen, or water (Free 1960; Robinson et al. 1984). Amdam, Csondes, et al. (2006) have argued that associations between foraging specialization and reproductive physiology (in particular the number of ovarioles in the ovary) indicate that the gene networks that now regulate worker foraging behavior first evolved to control the reproductive cycle of solitary insects. That is, pollen foragers tend to express the genes associated with the solitary ancestor's reproductive phase and nectar foragers express the gene networks associated with the foraging (nonreproductive) phase (Amdam et al. 2004; Amdam, Csondes, et al. 2006; Page et al. 2006; Tsuruda et al. 2008; Page 2013). The association between foraging specialization and reproduction is assumed to be

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general across social insects (Page et al. 2006; Page and Amdam 2007). We will refer to this hypothesis as the "Reproductive Ground Plan-forager" hypothesis (Oldroyd and Beekman 2008).

Another hypothesis linking foraging to reproductive traits can be described as the "Reproductive Conflict and Work" hypothesis (Schmid-Hempel 1990). This hypothesis argues that whenever there is the potential for reproductive conflict among workers, workers that avoid risky behavior like foraging are more likely to be successful in reproduction than workers that do forage. Modeling shows that there is likely to be a stable equilibrium between dominant workers that avoid foraging and are most likely to become reproductive when the opportunity arises, and subordinate workers that work hard and are least likely to reproduce (Schmid-Hempel 1990). In support of this hypothesis, there is widespread evidence that reproductive individuals contribute less to colony maintenance than nonreproductive individuals across social insects including bumble bees (Jandt and Dornhaus 2011), ants (Ito and Higashi 1991; Powell and Tschinkel 1999), and wasps (Cant and Field 2001).

Support for the Reproductive Ground Plan-forager hypothesis is mainly confined to honey bees. Genetic and behavioral studies on 2 strains of honey bee that have been selected for low and high pollen hoarding (Hellmich et al. 1985; Page et al. 2012) show that workers from the high pollen hoarding strain transition to foraging earlier in life, have more ovarioles within their ovaries, are more likely to activate their ovaries when in a queenless colony, and respond (by feeding) to nectar containing low concentrations of sucrose. Foragers of the low pollen hoarding strain transition to foraging later in life, have a smaller number of ovarioles, and respond only to high concentrations of sucrose (reviewed in Page et al. 2012; Page et al. 2006). These studies provide evidence for associations between ovariole number, reproduction, and foraging behavior. Studies on wild-type bees have revealed similar associations (Page et al. 2006; Page et al. 2012).

Further evidence supporting the Reproductive Ground Plan-forager hypothesis comes from a backcross between 2 honey bee subspecies *Apis mellifera ligustica* and *Apis mellifera scutellata* (Linksvayer et al. 2009). In this study, backcrossing generated a worker population with large variation in ovariole number, the extremes of which are not seen in either of the parental subspecies. This variation in ovariole number was significantly correlated with foraging specialization: workers that had a high number of ovarioles foraged preferentially for pollen, thus providing further evidence for a link between reproductive physiology and foraging behavior (Linksvayer et al. 2009).

More direct evidence for an association between ovary size, synthesis of the egg yolk protein vitellogenin in the fat body, and foraging specialization comes from manipulative studies. Knockdown of vitellogenin synthesis increased the proportion of workers that collected nectar relative to the proportion that collected pollen (Nelson et al. 2007). Surgically increasing the ovarian mass of workers by injecting ovaries into the worker's abdominal cavity leads to onset of foraging at an earlier age and specialization in pollen collection (Wang et al. 2010). These studies provide experimental rather than correlative evidence that reproductive physiology and the number of ovarioles are causally linked with foraging behavior in the honey bee (Wang et al. 2012).

Despite the extensive evidence supporting the Reproductive Ground Plan-forager hypothesis, there are both theoretical and empirical reasons to doubt its generality. First, Johnson and Linksvayer (2010) point out that task allocation in insect colonies needs to be responsive to changes in colony demography and in the environment. Thus, a character like ovariole number, which is fixed for adult life (Schmidt Capella and Hartfelder 1998), is unlikely to be the primary determinant of foraging behavior. Second, workers of a selected anarchistic strain show unusually high rates of worker reproduction, with up to 30% of workers having eggs in their ovarioles compared with less than 1% in wild-type strains (Barron et al. 2001). Based on the Reproductive Ground Plan-forager hypothesis, Oldroyd and Beekman (2008) hypothesized that workers of the anarchistic strain should have a high numbers of ovarioles, would forage early in life, have a preference for pollen over nectar, and would carry heavier pollen loads than wild-type workers. Contrary to these predictions, it was shown that when anarchistic and wild-type bees were fostered into the same wild-type colonies, anarchist workers began foraging later in life than wild-type workers (Oldroyd and Beekman 2008). Both anarchist and wild-type workers were equally likely to collect nectar or pollen, irrespective of their number of ovarioles. Moreover, workers that most likely never foraged had higher rates of ovary activation compared with workers that did forage, irrespective of their genotype (anarchistic or wild type). It was therefore concluded that the gene networks regulating reproductive physiology and foraging specialization are not causally linked (Oldroyd and Beekman 2008). In addition, wild-type workers had more ovarioles than workers from the anarchistic line, casting doubt on the assumption that number of ovarioles is positively correlated with reproductive ability of workers as previously reported (Amdam, Csondes, et al. 2006; Makert et al. 2006).

Oldroyd and Beekman (2008) argued that their study of the anarchist strain selected for high rates of worker reproduction supported the original Ground Plan hypotheses (West-Eberhard 1996; Hunt and Amdam 2005), but not its later extension to the Reproductive Ground Plan-forager hypothesis. They further suggested that their study supported the Reproductive Competition and Work hypothesis, as workers most likely to be reproductively active were least likely to engage in risky foraging behavior.

Oldroyd and Beekman's (2008) study as well as some studies supporting the Reproductive Ground Plan-forager hypothesis (Amdam, Csondes, et al. 2006; Linksvayer et al. 2009) can be criticized on the grounds that they are based on behavior and physiology of genotypes that are artificially generated (Amdam and Page 2008). Selection for pollen hoarding or reproductive behavior, or creating a backcross between subspecies, may have indirect effects on behavior and physiology that are not normally found in nature. Ideally, the RGP-forager hypothesis should be tested in a population that has not been subjected to artificial selection or crossing but shows high variation in ovariole number in workers. The sexual, nonparasitic population of *Apis mellifera capensis* from South Africa is ideal for this purpose.

Apis m. capensis is only found in southern South Africa (Hepburn and Crewe 1991). Uniquely among honey bee subspecies, many unmated workers lay diploid female-destined eggs by thelytokous parthenogenesis (Verma and Ruttner 1983). Workers from all other subspecies can only produce haploid male-destined eggs by arrhenotokous parthenogenesis. Though *A. m. capensis* workers are normally sterile (Beekman et al. 2009), in queenless colonies they lay diploid eggs in queen cells of their own or other colonies that go on to develop into queens (Jordan et al. 2008; Allsopp et al. 2010; Holmes et al. 2010; Moritz et al. 2011). Thus, *A. m. capensis* workers have been selected for higher reproductive potential than workers of other species, and workers show much greater variance in reproductive traits than other subspecies (Hillesheim et al. 1989). *Apis*

m. capensis workers have 10–20 ovarioles per ovary (Goudie et al. 2012) compared with 1–6 ovarioles in other subspecies (Amdam, Csondes, et al. 2006) and often possess a spermatheca (Ruttner 1988), a sperm storage organ normally only found in queens.

On the assumption that ovariole number and presence of a spermatheca are measures of reproductive potential and that ovarioles directly modulate foraging preference, the Reproductive Ground Plan-forager and Reproductive Conflict and Work hypotheses make clear and often contrary predictions about these physiological attributes and the foraging behavior of *A. m. capensis* workers (Table 1). Here we examine these predictions in a population of unselected *A. m. capensis*. In doing so we test 2 fundamental but somewhat opposing hypotheses concerning the evolution of eusociality and task specialization in eusocial insects. In addition, we test the assumption that the number of ovarioles is a reliable proxy of a worker's reproductive potential by investigating the relationship between ovariole number and ovary activation.

MATERIALS AND METHODS

Ovariole number and ovary activation

The study was conducted at the Plant Protection Research Institute in Stellenbosch, South Africa. In December 2011, we removed brood combs containing emerging brood from each of 3 colonies of *A. m. capensis* and individually caged the combs. All colonies were open-mated and unrelated and representative of the natural *A. m. capensis* population. The combs were incubated overnight at 35 °C and high relative humidity. Emerging workers were paint-marked using Posca Posta Pens (Mitsubishi Pencil Co. Japan) over 2 days, so that the emergence date and colony of origin were discernible. We introduced the marked workers into a fourth *A. m. capensis* colony that had been made queenless 2 days earlier. Throughout this experiment we removed any queen cells that developed.

We sampled up to 100 workers per source colony when the workers were 5, 8, and 11 days of age. Sampled workers were frozen at −20 °C until dissection to determine the degree of ovary activation and ovariole number. To dissect a worker, we pinned it to a wax plate through the thorax. While irrigating with water, the abdomen was pulled apart between the third and fourth tergite using dissecting forceps. The right and left ovaries were then removed using dissecting scissors and placed into separate drops of water on a glass slide, which were then covered with coverslips. After applying slight pressure to the coverslip to spread the ovaries, we counted the number of ovarioles. Where an ovary could not be located we recorded the ovariole number of the single ovary.

The degree of ovary activation was scored on a 4-point scale (Velthuis 1970): 1 = tiny thread-like transparent ovarioles, 2 = thickened and opaque but with no evidence of segmentation, 3 = the ovarioles were segmented but the eggs were not fully formed, and 4 = fully formed white eggs.

Age at first foraging

In January 2009, we removed combs of emerging workers from 2 *A. m. capensis* colonies and incubated them overnight, as described above. Workers were paint-marked according to their colony of origin and introduced into a third, queen-right colony that had a normal demographic distribution of nurse bees and foragers. The host colony was housed in a 4-frame nucleus hive that had been fitted with an entrance tunnel to facilitate capture of marked foragers as they returned from foraging trips.

After the introduced workers were 5 days old, the host colony entrance tunnel was observed daily between 9 AM and 1 PM until the marked workers were 17 days old. As a marked worker entered the entrance tunnel, a gate was dropped so that she could not enter the colony. The returning paint-marked forager was then grasped with forceps and placed in a labeled microcentrifuge tube on ice to cool her to immobility. This procedure likely catches workers on one of their first orientation flights (Capaldi et al. 2000) but is generally regarded as the "age at first foraging" (Amdam, Csondes, et al. 2006).

This procedure was replicated using another 2 source colonies and an independent host colony in December 2012.

Foraging preference

We introduced day old workers from 2 source colonies into a single host colony. When workers were 21 days old, we collected them as they returned from foraging trips until we had a sample of 100 from each source colony and determined the volume and concentration of dissolved nectar and the weight of the pollen carried by each returning forager (Oldroyd and Beekman 2008). We then froze the bees prior to dissection to determine the number of ovarioles.

When the remaining introduced workers were 29 days old, we moved the host colony 20 m away from its original site and replaced it with a decoy hive that contained combs of capped brood but no adult bees. As workers left the host colony and foraged, they returned to the decoy hive on the original site. After 6 h, we opened the decoy hive and collected all marked foragers. We also collected all marked "nonforagers" from the original host on its new site. We made the assumption that the marked workers that had remained in the original colony had not foraged, for if they had they would have returned to the original site that they had learned during

Table 1

Predictions concerning the association between reproductive traits and foraging behavior in *Apis mellifera capensis*

Association	Reproductive Ground Plan-forager hypothesis	Reproductive conflict and work hypothesis
Correlation between age at first foraging and ovariole number	Negative	Positive
Correlation between ovariole number and tendency to collect pollen	Positive	No association
Correlation between ovariole number and dissolved sugar in nectar loads	Negative	No association
Association between ovariole number/ presence of spermatheca and tendency to forage	Positive	Negative

previous foraging trips (Oldroyd and Beekman 2008). These bees were also frozen prior to dissection.

We dissected the frozen bees and scored them for ovary activation and ovariole number as described above. We also recorded the presence or absence of a spermatheca, a character that is correlated with worker reproduction (Hepburn and Crewe 1991).

RESULTS

Ovariole number and ovary activation

Across the combined age classes and 3 source colonies, there was a significant negative association between ovariole number and ovary activation as measured using Velthuis's (1970) 4-point scale (Spearman's $\tau = -0.19$, $n = 767$, $P < 0.001$). This negative association was present for workers aged 5 days ($\tau = -0.23$, $n = 289$, $P < 0.001$) and 11 days ($\tau = -0.23$, $n = 194$, $P < 0.001$) but not when they were aged 8 days ($\tau = 0.048$, $n = 284$, $P = 0.42$). Across combined ages and colonies and based on a mixed model, workers with activated ovaries (scores 3 and 4) had significantly fewer ovarioles than workers with inactive ovaries (scores 1 and 2) (Figure 1, fixed effect, $F_{1,2.04} = 28.38$, $P = 0.032$). There was no significant effect of source colony on ovariole number (random effect, $F_{2,0.52} = 30.84$, $P = 0.29$), and no significant interaction between source colony and ovary score on ovariole number ($F_{2,4.23} = 1.35$, $P = 0.35$).

Age at first foraging

We found a weak but significant positive correlation between ovariole number and age at first foraging ($\tau = 0.11$, $n = 697$, $P = 0.002$), indicating that workers with more ovarioles tended to forage later in life than workers with fewer ovarioles. Because of the possibility of truncation bias, we followed Amdam, Csondes, et al. (2006) and divided our sampled workers into 3 roughly equal bins of ovary size 1–12, 13–17, and 18+ ovarioles and determined if ovariole number had an effect on age at first foraging using 2-way Anova of ovariole-number bin and colony as a random effect. Although there was no significant effect of ovariole bin overall ($F_{2,7.4} = 1.32$, $P = 0.32$) or source colony ($F_{3,6.08} = 4.19$, $P = 0.06$), workers with the smallest number of ovarioles foraged at a significantly younger age than workers with the largest number of ovarioles (Figure 2).

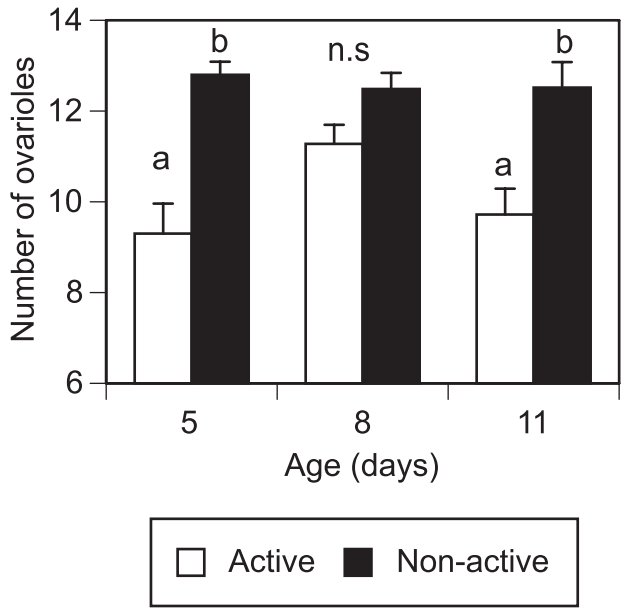

Figure 1
Number of ovarioles in pooled workers from 2 source colonies with nonactive and active ovaries in a queenless colony at 5 ($n = 289$), 8 ($n = 284$), and 11 ($n = 194$) days of age. Error bars are standard errors of the means. Within each age group, bars with a different letter are significantly different ($P < 0.05$, LSD following Anova of source colony and ovary size class). n.s. = not significantly different.

Foraging preference

We had allowed a second cohort of workers to mature for 3 weeks. For these experienced foragers, we examined the relationship between ovariole number and the loads which foragers carried. Among the 209 bees captured, there was no significant correlation between ovariole number and pollen weight ($\tau = 0.04$, $P = 0.53$), volume of crop contents ($\tau = -0.015$, $P = 0.83$) or nectar concentration ($\tau = 0.024$, $P = 0.73$). When we considered only the 128 bees that carried liquid, there was no significant association between ovariole number and the volume ($\tau = 0.02$, $P = 0.84$) or concentration ($\tau = 0.01$, $P = 0.94$) of the crop contents (Figure 3). When we considered the 76 bees that carried pollen, there was no significant correlation between the number of ovarioles and the weight of pollen carried (Figure 3, $\tau = -0.104$, $P = 0.37$). Bees that carried pollen had similar numbers of ovarioles (11.97 ± 5.2) to bees that did not (12.14 ± 4.7) ($F_{1,207} = 0.06$, $P = 0.81$), and there was no significant interaction between forager type (pollen forager vs. nonforager) and source colony ($F_{1,205} = 0.31$, $P = 0.58$).

Mature (4 week old) workers that had remained in the nest and most likely had never foraged had significantly fewer ovarioles (11.44 ± 0.77) than workers that had foraged (13.54 ± 0.65) ($F_{1,304} = 4.33$, $P = 0.038$). Nonforagers had a higher rate of ovary activation than the foragers (Figure 4, $\chi^2_2 = 44.39$, $P < 0.001$) and significantly more nonforagers had a spermatheca than did foragers (Figure 4, $\chi^2_1 = 13.03$, $P < 0.001$).

DISCUSSION

The Reproductive Ground Plan-forager hypothesis argues that ovariole number influences foraging behavior because larger

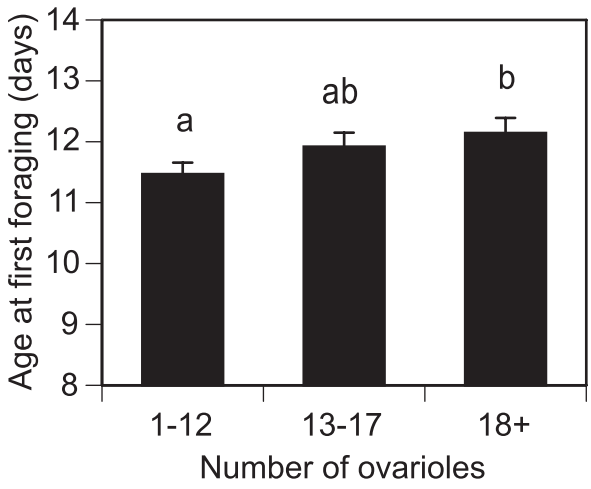

Figure 2
The relationship between ovariole number and age at first foraging. Bars with different letters are significantly different ($P < 0.05$, LSD following 2-way Anova of source colony and ovary size class). Error bars are standard errors of the means. 1–12 ovarioles, $n = 216$; 13–17, $n = 219$; 18+, $n = 218$.

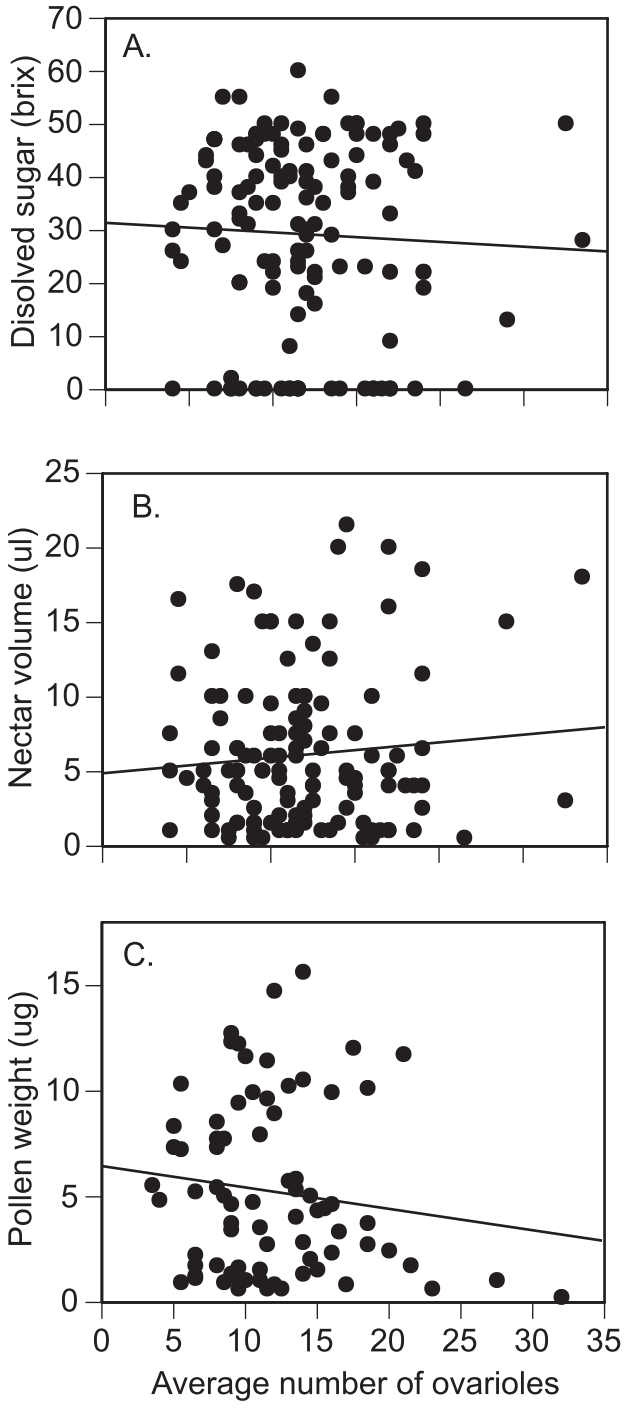

Figure 3
The relationship between average ovariole number and forager loads in 3-week-old *Apis mellifera capensis* foragers from 2 colonies cross-fostered into a single host colony. Lines are the linear regressions of the indicated measure on ovariole number. There is no significant correlation between ovariole number and any of the measured foraging traits (see text). (A) The amount of dissolved sugar (brix) in the crop contents of the 128 workers that carried nectar or water. Slope of the regression line, $\beta = -0.18$, $R^2 = 0.002$. (B) The volume (μL) of nectar/water carried by the 128 returning foragers that carried liquids $\beta = 0.010$, $R^2 = 0.0082$. (C) The mass of pollen carried by the 76 foragers that carried pollen $\beta = -0.10$, $R^2 = 0.017$.

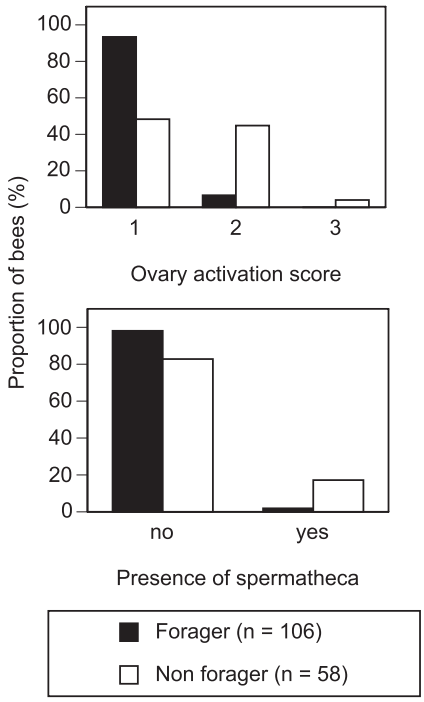

Figure 4
Reproductive traits of mature workers that had foraged and that had probably never foraged. Workers that did not forage had significantly higher rates of ovary activation and were significantly more likely to have a spermatheca than workers that foraged (see text).

ovaries signal for greater synthesis of the yolk precursor protein vitellogenin in the fat body and that vitellogenin either directly or indirectly affects sucrose concentration responsiveness, which in turn affects foraging behavior (Amdam et al. 2004; Amdam, Norberg, et al. 2006; Nelson et al. 2007; Tsuruda et al. 2008; Amdam and Page 2010; Ihle et al. 2010). Under this assumption, the Reproductive Ground Plan-forager hypothesis predicts that workers with more ovarioles should forage early in life and preferentially forage for pollen, whereas workers with fewer ovarioles should forager later in life and specialize in nectar (Table 1). Contrary to these predictions, we found no significant association between ovariole number and foraging specialization. Instead of a negative correlation between number of ovarioles and age at first foraging, we found a positive correlation. Thus, our data do not support the Reproductive Ground Plan-forager hypothesis.

A similar positive (but not significant due to small sample size) association between the number of ovarioles and age of first foraging has been reported in the related honey bee species *Apis cerana* (Rueppell et al. 2008) and in the "anarchist" strain selected for high rates of worker reproduction (Oldroyd and Beekman 2008). Thus, 3 independent studies using widely different populations of *Apis* have suggested that, if anything, bees with more ovarioles start foraging at an older age than workers with a smaller number of ovarioles. We suggest that this positive association with age at first foraging occurs in populations where workers are actively reproductive and that in such populations workers that have activated ovaries delay foraging. Thus, previous findings where the number of ovarioles is negatively correlated with age at first foraging are from wild-type *A. mellifera* that are normally sterile (Amdam, Csondes, et al. 2006)

and do not hold in populations in which workers are actively reproductive in the presence of a queen.

Our data also suggest that in populations where workers are routinely reproductively active, a large number of ovarioles does not equate to high reproductive success. We found that workers that remained in the nest and probably never foraged had fewer ovarioles than workers that foraged, and those workers were more likely to have swelling of the ovarioles or carry eggs in their ovaries. Moreover, among queenless workers, there was a negative association between ovariole number and ovary activation scores. A positive association between ovariole number and ovary activation has been repeatedly reported (Amdam, Csondes, et al. 2006; Makert et al. 2006; Oldroyd and Beekman 2008). Thus, our finding of a negative association between the number of ovarioles and ovary activation in queenless *A. m. capensis* workers was surprising**.** Nonetheless, such an association has been noted previously in *A. m. capensis* (Allsopp 1988).

Ironically, the negative association between ovary size and ovary activation observed in *A. m. capensis* could be construed as support for the Reproductive Ground Plan-forager hypothesis, as bees with higher reproductive potential (i.e., smaller number of ovarioles) initiated foraging at an earlier age. However, a central tenant of the Reproductive Ground Plan-forager hypothesis is that the number of ovarioles and the signals the ovary mediates via vitellogenin synthesis in the fat body regulates foraging preference. Such a link is not present in *A. m. capensis* workers, nor in the anarchistic honey bee strain. Thus, a test of the assumption critical to the Reproductive Ground Plan-forager hypothesis fails in populations with reproductively active workers.

In our study, mature workers that we assumed did not forage or foraged less were more likely to have activated ovaries and a spermatheca than workers that did forage. This suggests that workers with a predisposition toward reproduction are less, not more, likely to forage than workers with a lower tendency to reproduction. In support of this view, bidirectional selection for reproductive "dominance" and "subordinance" in *A. m. capensis* workers generated strikingly different behavioral phenotypes (Hillesheim et al. 1989). Subordinate workers were more likely to offer food than dominant workers. When colonies comprised workers of the "dominant" line, the colonies performed poorly, whereas colonies comprised workers of the "subordinate" line performed well in worker tasks like comb building, brood rearing, and hoarding food. "Dominant" workers are risk adverse, unlikely to work, and had more activated ovaries and produced more queen-like pheromones than "subordinate" workers (Moritz and Hillesheim 1989). These observations give further support to our contention that reproductive workers are less, not more, likely to forage than sterile workers. They provide greater support for the Reproductive Conflict and Work hypothesis (Houston et al. 1988; Schmid-Hempel 1990) than for the Reproductive Ground Plan-forager hypothesis.

There is now independent evidence from a highly reproductive subspecies and from a line selected for high rates of reproduction, the number of ovarioles is not causally linked to foraging specialization in *A. mellifera*. These findings falsify a clear prediction of the Reproductive Ground Plan-forager hypothesis as it is currently postulated. Thus, we suggest that while the original Ground Plan hypothesis (West-Eberhard 1996) is undoubtedly a useful paradigm for examining the mechanistic origins of eusociality, its extension to understanding the foraging behavior of modern honey bees based on an association with ovariole number via production of vitellogenin is questionable because its key predictions are not always supported.

Hunt and Amdam (2005) have emphasized that the evolution of eusociality is best understood by examining the "regulatory circuits" present in solitary ancestors and the means by which these gene networks have been modified in the worker caste to new functions. They downplay examination of the selective benefits of conflict and cooperation in explaining how worker behavior is regulated. We argue that the 2 paradigms are not mutually exclusive. It is parsimonious that many of the genes that kin selection acts on once regulated the reproductive cycles of solitary ancestors. Nonetheless, it is becoming increasingly clear that the genomes of honey bees and social insects in general comprise large numbers of taxonomically restricted genes that are differentially expressed between the queen and worker castes (Johnson and Tsutsui 2011; Ferreira et al. 2013; Simola et al. 2013). Thus, the evolution of sociality did not rest on the modification of existing gene networks alone. Our study suggests that contemporary reproductive conflict remains a powerful driver in determining the foraging work that social insect workers perform, particularly with respect to the onset of foraging (Schmid-Hempel 1990).

FUNDING

This work was funded by an Australian Research Council grant DP0878924 to M.B. and B.P.O. and a University of Sydney sabbatical leave grant to B.P.O.

Handling editor: Anna Dornhaus

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